

Integrating WGCNA and PPI network to screen heat stress-responsive hub genes of *Pinellia ternata*

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Abstract

Background

Pinellia ternata (Thunb.) Breit. is a heat-sensitive herb. Heat damage can lead to leaf senescence and even death, but the impact on underground parts is not significant when the ambient temperature exceeds 30°C. *P. ternata* has a diversity of leaf types, however, the response strategies of different leaf types to high temperatures have not been thoroughly analyzed. This study aims to explore hub genes response to heat stress shared in two main leaf types of *P. ternata* based on integrated network analysis for improving planting measures.

Results

The ultrastructure, physiological indices, and photosynthetic fluorescence parameters were investigated, which indicated that the leaves of *P. ternata* were able to prevent the damage of photosynthetic structures, averted the accumulation of ROS, and sustained photosynthetic physiological responses under moderate heat stress. Serious heat stress activated the antioxidant enzyme activity systems to provide protective effects. However, the structure and function of chloroplasts in *P. ternata* leaves were adversely affected. By analyzing the transcriptome data, we obtained a total of 20,875 DEGs. Furthermore, weighted gene co-expression network analysis (WGCNA) was performed to explore the main modules related to heat stress, and 6,183 DEGs were obtained in five candidate modules. Among them, 1,000 DEGs could be annotated by the Uniprot and STRING databases, and a protein-protein interaction (PPI) network was constructed based on these DEGs. In this network, we identified 54 hub genes, and these genes were mainly related to thermal stimulation (HSPs, HSFs, and molecular chaperones) and photosynthesis (Photosystem I chlorophyll a/b-binding protein, Chlorophyll a-b binding protein et al.).

Conclusion

The response mechanisms to high-temperature treatment of two leaf types of *P. ternata* were analyzed at physiological, subcellular, and molecular levels. The results indicate that these two germplasms shared a common strategy in response to heat stress, and hub genes obtained provide valuable genetic resources for molecular resistance breeding in *P. ternata*.

Background

During the first half of the 21st century, the greenhouse gas effect is projected to cause a temperature increase of 2–5°C, significantly exacerbating the detrimental effects of heat stress on plants^[1]. Being sessile organisms, plants exhibit responses to high temperatures, including the inhibition of photosynthesis, leaf and stem wilting, scorching and abscission, ultimately leading to leaf senescence

[2]. *Pinellia ternata* (Thunb.) Breit., a temperature-sensitive medicinal plant, is mainly cultivated in open fields in China [3, 4]. *P. ternata* thrives at 18 ~ 25°C; high temperatures above 30°C can cause thermal severe damage to its leaves, resulting in stress-induced leaf senescence, sprout tumble and shortened growth period. Therefore, heat stress is one of the abiotic factors constraining the survival and yield of *P. ternata* [5–7].

The response mechanism of *P. ternata* under heat stress has a broad research basis spanning plant physiology and transcriptome. Exposure to heat stress induces leaf senescence in *P. ternata*, characterized by elevated levels of reactive oxygen species (ROS) and damage to the photosynthetic system [8–10]. Moreover, the leaf senescence in *P. ternata* is linked to programmed cell death, inferred from the alterations in cell physiology and morphology [11]. At the transcriptional level, several genes in *P. ternata* leaves were involved in photosynthesis, redox regulation, amino acid metabolism and signalling under heat stress [12]. Heat stress may impede the synthesis of secondary metabolites in the tubers of *P. ternata*, including the downstream products of the flavonoid synthesis pathway [13]. The gene expression of heat shock proteins (HSPs) and the stearoyl-ACP desaturase gene (SAD) in *P. ternata* was found to be up-regulated following high-temperature stress. These proteins and enzymes contribute to the maintenance of intracellular protein and lipid stability, thereby enhancing the adaptability to high temperatures [14–16]. The aforementioned studies preliminarily elucidated the regulatory mechanisms in response to heat stress in *P. ternata*. Previous studies have found that *P. ternata* exhibits a substantial range of leaf type diversity [17–19]. However, the mechanism in response to high temperatures of these genotypes still needs to be fully elucidated.

The aim of this study is to explore the physiological and molecular mechanisms underlying heat tolerance in two main leaf types of *P. ternata* and identify hub genes involved in this process using WGCNA and PPI network. The results offer valuable genetic resources for heat-induced molecular breeding in *P. ternata*.

Results

Phenotypic and physiological changes of TY and ZY leaves under different heat treatments

As the temperature gradually increased, TY and ZY remained green at 31°C (moderate heat stress). However, at 43°C (severe heat stress), TY and ZY yellowed and appeared to have significant leaf senescence phenotype (Fig. 1a and 1b). Under severe stress, the mesophyll cells incurred significant damage in both TY and ZY, such as protoplast deformation and compression, plasma membrane damage or rupture, and organelle disintegration (Figure S1). A significant concentration of osmiophilic particles with high electron density was found in the chloroplast (Fig. 1a and 1b). Both the grana lamellae and stroma lamellae were noticeably fractured, indicating serious damage to the inside of the chloroplast.

Both SOD and POD activities showed increasing trends, and there were no significant changes under moderate stress in TY31 and ZY31 compared to the control. Specifically, SOD activities of TY31 and ZY31 increased by 13.77% and 4.52%, respectively. POD activities increased by 8.86% and 12.86%, respectively. Notably, the activities of antioxidant enzymes increased significantly under severe heat stress (Fig. 1c). SOD activities of TY43 and ZY43 increased by 28.17% and 20.02%, respectively, while the POD activities increased by 127.85% and 195.71%.

Additionally, the relative expression of two marker genes associated with photosynthesis was analyzed (Fig. 1c). Under moderate heat stress, the expression of *PtLHC1* in TY31 and ZY31 was down-regulated, and the expression levels were reduced by 43.79% and 55.08%, respectively, compared with the control group. The relative expression of *PtCAB* in TY25 and ZY25 was 76.42% and 60.62%, which was lower than that in TY43 and ZY43, respectively. Under severe heat stress, the expression of *PtLHC1* in TY43 and ZY43 showed a down-regulated pattern, respectively 4.27 and 2.54-fold lower than that in the control group. The relative expression levels of *PtCAB* of TY43 and ZY43 were 31.71 and 23.55-fold lower than in the control group.

In order to explore the photosynthetic damage of *P. ternata* leaves under heat stress. The photosynthetic gas exchange and chlorophyll fluorescence parameters of TY and ZY were measured. The net photosynthetic rate (*A*) of the control group (TY25 and ZY25) was 8.21 and 8.98 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. However, this index decreased by 29.2% and 31.4% at 31°C and decreased by 86.1% and 81.1%, respectively, at 43°C. The transpiration rate (*E*) decreased by 11.9% and 8.3% at 31°C. At 43°C, they decreased by 73.4% and 64.4%, respectively. The photochemical efficiency (*Fv/Fm*) of photosystem II (PSII) is a crucial index for studying the impacts of photoinhibition and environmental stresses on photosynthesis. When exposed to severe heat stress, *Fv/Fm* of TY and ZY declined by 5.8% and 5.6% in TY43 and ZY43, respectively. The photochemical efficiency of PSII (ΦPSII) decreased significantly by 48.1% and 69.5%. The net photosynthetic rate (*A*), transmittance rate (*E*), photochemical efficiency (*Fv/Fm*) and photochemical efficiency of PSII (ΦPSII) of TY31 and ZY31 all decreased with almost no significant changes. Under severe stress, these parameters decreased significantly compared with the control group. The photosynthetic physiological responses of the two leaf types of *P. ternata* exhibited similar trends (Fig. 1c).

Five heat-related modules obtained by weighted co-expression network analysis

This study aimed to deepen understanding of the response to severe heat stress in *P. ternata* at the transcriptional level. Our analysis yielded 510,427,032 clean reads, with Q20 and Q30 values exceeding 97.4% and 93.1%, respectively (Table S1). The average GC content was 52.18%. The PCA analysis revealed strong consistency among three biological replicates of TY25, TY43, ZY25, and ZY43 (Figure S2a), with significant transcriptomes overlap. We conducted a correlation clustering analysis to depict the correlation between samples. The analysis results showed a good correlation between the expression levels of the four sample groups. A total of 20,875 differentially expressed genes (DEGs)

were obtained by combining the DEGs from TY25 vs TY43 and ZY25 vs ZY43 (Table S2). These DEGs were then subjected to cluster analysis to evaluate transcription abundance across four distinct groups in *P. ternata* (Figure S2b). In order to further analyze DEGs, WGCNA was used to find the interested modules from the modules that responded to heat treatment and analyze the gene functions. We filtered the data of 20,875 DEGs twice in the WGCNA package and obtained 10,419 DEGs (Table S3). Then, the 10,419 DEGs of 12 samples of *P. ternata* were significantly clustered into two categories: the heat treatment group and the control group (Fig. 2a). We determined the optimal power value for analysis to be seven, which resulted in an independence score of 0.8 and higher average connectivity. Twenty-three distinct co-expression modules were identified in *P. ternata* (Fig. 2b). The relationships between modules and heat stress are depicted (Fig. 2c), and five gene modules exhibited the strongest correlation with the target traits (turquoise, blue, magenta, midnight blue, and salmon).

The five gene modules were defined as the interested modules, and their functions were elucidated using KEGG analysis (Fig. 3). It showed that genes in the turquoise module were significantly enriched in photosynthesis-antenna proteins (ko00196), photosynthesis (ko00195), porphyrin metabolism (ko00860), and carbon fixation in photosynthetic organisms (ko00710). Genes in the blue module were enriched in spliceosome (ko03040), plant hormone signal transduction (ko04075), and protein processing in the endoplasmic reticulum (ko04141). In the magenta module, the significantly enriched pathways were flavonoid biosynthesis (ko00941) and brassinosteroid biosynthesis (ko00905). In the midnight blue module, genes were found to be significantly enriched in purine metabolism (ko00230), while in the salmon module, genes were enriched in valine, leucine, and isoleucine biosynthesis (ko00290) (Table S4). These pathways are closely related to the damage of leaves and the chlorophyll synthesis in *P. ternata*.

Identification of hub genes by protein-protein interaction (PPI) network analysis

There were 6,183 DEGs in the five modules above of interest. In this gene set, 1,000 DEGs could be annotated by the Uniprot and STRING databases, and these were utilized to construct the protein-protein interaction (PPI) network (Table S5-6). The PPI network was constructed using Cytoscape plugins (Cytoscape, CytoHubba, and MCODE) for visualization. A cluster with 417 nodes and 1,410 edges was identified by MCODE, employing a scoring system with a cut-off k-score of 12. This study further identified regions and communities within complex networks with a higher node and edge clustering density, potentially representing functional modules with biological implications. The MCC algorithm implemented through the CytoHubba plugin was utilized to identify proteins with a higher degree, and the top 30 proteins were filtered to elucidate hub proteins (Table S7). The findings indicated that the top 30 protein-protein interactions formed two functional modules associated with heat stress proteins and photosynthesis proteins, respectively. Specifically, the heat stress proteins functional module included 14 proteins (HSPs, HSFs, and molecular chaperones) encoded by 24 hub genes. Notably, eight genes encoding HSPs in TY43 were up-regulated, while all genes encoding HSPs in ZY43 showed up-regulation.

Furthermore, the photosynthesis functional module contained 16 proteins (Photosystem I chlorophyll a/b-binding protein, Chlorophyll a-b binding protein et al.) encoded by 30 hub genes (Fig. 4a and 4b).

Discussion

In this study, following a 12-hour exposure of TY and ZY leaves to high temperature stress, *P. ternata* was tolerant to moderate heat stress. However, under severe heat stress, the leaves of TY43 and ZY43 exhibited an obvious senescence phenotype. Changes in antioxidant enzyme activity, photosynthetic parameters, marker gene expression, and leaf structure damage were used to assess the response of TY and ZY to heat stress. The results indicated a similar change trend between TY, ZY and the previously studied *P. ternata* with peony leaf type ^[20].

Advancements in transcriptome sequencing technology and enhancements to gene function databases contribute significantly to investigating of plant stress tolerance mechanisms. Weighted gene co-expression network analysis (WGCNA) is a significant approach for elucidating the relationship between genes and treatment groups. It enables the identification of gene modules closely linked to the targeted treatment group, containing genes correlated with the specific treatment. In this study, the combined uses of WGCNA and PPI network allowed for screening the most critical genes in response to heat stress and, thus, the exploration of detailed mechanisms. TY and ZY were subjected to heat stress to establish a WGCNA co-expression network. KEGG pathway annotation was carried out for five interested modules, such as photosynthesis (ko00195) in the turquoise module, and protein processing in the endoplasmic reticulum in the blue module (ko04141). In previous studies, heat stress up-regulated most heat shock protein-related genes, which could maintain the stability of biomembrane and protein structure and protect intracellular protein from stress damage; Photosynthesis-related genes will be significantly down-regulated. In addition, heat stress predominantly enriched flavonoid biosynthesis ^[21, 22]. These are consistent with our analysis results. These enriched functions are consistent with the molecular response of *P. ternata* to heat stress. Protein-Protein Interaction (PPI) network is composed of proteins that interact with each other to participate in all aspects of life processes, such as gene expression regulation, energy and substance metabolism ^[23]. Twenty-four genes related to HSP, HSF and molecular chaperone and 30 genes related to photosynthesis were screened out. Seven genes related to heat shock proteins and twelve related to photosynthesis have been previously identified. The results indicated their potential significance in understanding the response to heat stress of *P. ternata* ^[20, 24].

Recently, there has been a growing suggestion that heat shock proteins play a crucial role in the molecular mechanism of *P. ternata*'s response to heat stress ^[25]. In our analysis, nine hub genes encode HSP70, HSP70-3, HSP70-8, HSP70-14, HSP70-15, HSP70-16 and HSP70-17, which exist in chloroplasts and mitochondria. In response to high temperature stress, they upregulated to meet the increased demand, as in *Arabidopsis*, where upregulation occurs at 37°C and 41°C for 30 minutes ^[26]. The rapid response of HSP70 may make it an essential gene, as suggested by previous studies. Hsp70s are vital components that facilitate the folding of de novo synthesized proteins, assist the translocation of

precursor proteins into organelles, and are responsible for the degradation of damaged proteins under stress conditions. It is likely more closely related to signal transduction, cellular hypoxia, and redox response^[27–28]. We focused on the analysis of the HSP70-14 gene, which is correlated to HSP 70 – 14, which has the most significant differential expression multiple. Silico expression analysis of publicly available microarray data revealed that *AtHSP70-14* is expressed ubiquitously in nearly all organs and developmental stages, with an upregulated transcript signal following heat shock. It has been observed that *AtHsp70-14/15* silenced plants are more sensitive to heat treatment, with only 9% of plants surviving the recovery phase after being subjected to heat stress at 38°C for five days. As previously discussed, *AtHsp70-14/15* was crucial in cellular adaptation to heat stress^[29]. HSP70 proteins require association with members of the HSP40 family, which are 40 kDa heat shock proteins, to fulfill their function^[26]. This dependency on HSP40 family members is an essential consideration for researchers studying the response of *P. ternata* to heat stress. Further investigation into the interactions between the HSP70 and HSP40 families could yield valuable insights.

Additionally, five hub genes (*Hsp90 family*, *HSP 90 – 5*, *HSP 90 – 6*, et al.), present in chloroplasts and mitochondria, were identified in our study. HSP90-5 Cooperates with TIC components and other molecular chaperones to drive the transport of preproteins into chloroplasts and functions in the chloroplast stroma to facilitate membrane translocation during protein import into the organelle^[30]. HSP90-6 stabilizes unfolding protein intermediates and functions as a folding molecular chaperone^[31, 32]. The HSP90 subfamily is closely linked to plant hormone secretion and signal transduction. Several metabolic pathways respond to high temperature stress, involving growth hormone, cytokinin, JA, and oleuropein lactone^[33]. These hub genes need to be validated further.

Conclusions

This study analyzed the response mechanism of two leaf types of *P. ternata* to heat treatment at physiological, subcellular, and molecular levels. Both genotypes were found to share a common response strategy to heat stress. Integration of WGCNA and PPI network facilitated the identification of hub genes; in-depth analysis of these genes will deepen our understanding in response to heat stress of *P. ternata*.

Materials and Methods

Plant materials and treatments

Tubers of *P. ternata* used in this study were supplied by Hubei Jiuzhou Jinbanxia Agricultural Comprehensive Development Co., Ltd. and identified by Prof. Chen Shi-Lin of the Institute of Chinese Materia Medica at the China Academy of Chinese Medical Sciences. Two *P. ternata* leaf type cultivars, namely TY (peach leaf type) and ZY (bamboo leaf type), were employed. These materials were considered excellent germplasm with high levels of alkaloids and organic acids^[28]. The voucher

specimen has been deposited at the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. Tubers with a dry, clean epidermis and free from insects were meticulously selected for this study. Cylindrical plastic pots, 15 cm in diameter and 20 cm in height were chosen to accommodate six tubers each. These pots were filled with soil from the *P. ternata* planting base in Zhongxiang County and placed on the experimental field at the Medicinal Botanical Garden of Hubei University of Chinese Medicine. The pots were 10 cm deep, offering sufficient space for the tubers to grow. The plants were grown outdoors for 40 days using local traditional cultivation practices and adequate moisture to ensure maturity. Subsequently, the plant materials were exposed to various temperatures (25°C, 31°C, and 43°C) for 12 hours. Leaf samples (TY25, TY31, TY43, ZY25, ZY31 and ZY43) were collected to analyse of antioxidant enzyme activity, photosynthetic parameters, ultrastructure, and transcriptomics.

Determination of antioxidant enzyme activity

To prepare the sample, 0.2 g fresh *P. ternata* leaves were collected and placed in a 10 mL tube. Next, we added 2 mL of pre-cooled phosphoric acid buffer, which contained 1% polyvinylpyrrolidone (PVP), 50 mmol·L⁻¹, and had a pH of 7.8. We then ground the samples into homogenates using an ice bath. To ensure that all of the sample was collected, we rinsed the mortar three times with additional phosphoric acid buffer until the final volume reached 5 mL. The homogenate was subjected to centrifugation at 10,000 rpm for 15 min at 4°C. The supernatant was used for the determination of SOD and POD activities, and the method of determination was referred to in the literature ^[34, 35].

Measurements of photosynthetic parameters

The photosynthetic parameters of *P. ternata* after heat treatment were measured using a portable photosynthetic apparatus (LI-COR, USA, Li-6800). Circular leaf chambers (2 cm²) were used to fill the leaves completely while avoiding the veins during clamping. Three plants were measured for each treatment. The leaf chamber was subjected to a light intensity of 1100 μmol·m⁻²·s⁻¹, while the blade chamber maintained a CO₂ concentration of 400 μmol·mol⁻¹ using a steel cylinder (ISI, Austria, Soda Chargers). The chamber's relative humidity was maintained at 55%, and the gas flow rate was set at 500 μmol·s⁻¹. To measure gas exchange parameters, we recorded the net photosynthetic rate (*A*), intercellular CO₂ concentration (*C_i*), transpiration rate (*E*), and stomatal conductance (*G_s*). In this study, we measured various chlorophyll fluorescence parameters under optimal light intensity. These parameters included the quantum yield of photosystem II (*Φ*PSII), non-photochemical quenching (NPQ), and electron transfer rate (ETR) of PSII. To assess the maximum photochemical efficiency (*F_v/F_m*) under dark conditions, we wrapped the same leaves in tin foil for 20 minutes of dark adaptation.

Observations of leaf ultrastructure

The middle compound leaves were collected from the plant materials and promptly immersed in a 2.5% (v/v) glutaraldehyde solution prepared with pre-chilled 0.1 M PBS. Next, we cut the leaves into small pieces measuring 5 mm×5 mm and placed them into 2 mL centrifuge tubes. After fixation for over 2

hours, we rinsed the leaf segments with PBS three to four times. The samples underwent dehydration using graded ethanol solutions and were then embedded in Epon812 epoxy resin (SPI Company, Batch No. 90529-77-4). The resulting blocks were then sectioned into ultra-thin slices using UC7 technology (Leica, Germany). The grids were stained with uranyl acetate and lead citrate before being examined using an HT7800 transmission electron microscope (Hitachi, Japan).

RNA extraction and qRT-PCR analysis

The RNA Easy Fast Plant Tissue Kit (TIANGEN BIOTECH (BEIJING) CO., LTD., DP452) was employed for total RNA extraction from the collected leaves. Then, the quality and purity of the extracted RNA were evaluated using 1% agarose gel electrophoresis and NanoDrop™ 2000 spectrophotometer (Thermo Fisher, USA). The qRT-PCR analysis (Thermo Fisher 7500) was utilized to analyze the relative expression levels of *PtLHC1* and *PtCAB* in various samples. *PtGAPDH* was selected as the internal reference gene, and three biological replicates were performed for each sample. The relative expression levels of different genes were calculated using the $2^{-\Delta\Delta Ct}$ method [36]. The relevant gene primers are provided in Table S10.

Transcriptome data analysis

The raw images were processed into raw reads and refined by removing chimeric sequences, polynucleotide sequences, and low-quality reads. Trinity V2.6.6 was employed to generate unigenes. Differential expression analysis was conducted using DESeq2, with a threshold value of q_1 . FPKM values were utilized to determine the relative expression level of DEGs. KEGG enrichment analysis of DEGs was performed using ClusterProfiler software. The criterion was established for significant differences in gene expression as q -value (FDR) < 0.05 and $|\log_2 \text{FoldChange}| \geq 2$.

WGCNA Analysis

This study evaluated the WGCNA algorithm for DEG expression, and TBtools v1.09876 was used for WGCNA analysis [37]. After background correction and quantile normalization, we selected the top 20% of genes (10419 genes) with the most variation in the analysis of variance for further analysis. Based on the scale-independent conditions, a power value of 7 was deemed appropriate. We employed the dynamic TreeCut algorithm with a minimum module size of 30 to identify the modules. Modules were selected with a correlation coefficient of $\geq |0.75|$ and a p -value of ≤ 0.01 for further analysis. The relationship between the modules was then summarized by analyzing the hierarchical clustering tree diagram of the characteristic genes and the heatmap of the corresponding characteristic gene network [38]. DAVID databases (DAVID, <https://david.ncifcrf.gov/summary.jsp>) and KEGG Orthology Based Annotation System (KOBAS, <https://bio.tools/kobas>) were used to perform enrichment analysis for the key modules containing the most genes. The analysis results were extracted under $p < 0.05$ after correction.

Protein-Protein Interaction (PPI) network construction

PPI Network and Cytoscape tool v3.10 to create and display protein-protein interaction network. First, we import unigenes from various modules annotated by Uniprot into the STRING database (<http://string-db.org/>) to establish the PPI relationship between these unigenes in different modules. Next, these PPI relationships were used for visualization by the Cytoscape v3.10^[39]. The module's top 10% of the most connected genes were classified as hub genes.

Statistical analysis

Statistical analysis in this study was performed using GraphPad Prism 8.0 software. The data were expressed as mean $\pm$ Se, and one-way ANOVA was employed for group comparisons. Significant differences between the groups were considered at p -values < 0.05 , 0.01 , and 0.001 .

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The transcriptome data generated in this study are deposited in the NCBI SRA database under BioProject PRJNA1073242. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material; further inquiries can be directed to the corresponding author/s.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' Contributions

J.L.W. conceived and designed the project. J.L.W., and J.L.C. performed the experiments. J.L.C., Z.M.Y. and L.L. analyzed the data. J.L.W. wrote the manuscript. X.Y.Z., X.F. and X.W.L. supervised and revised the manuscript. All authors read and approved the manuscript.

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Figures

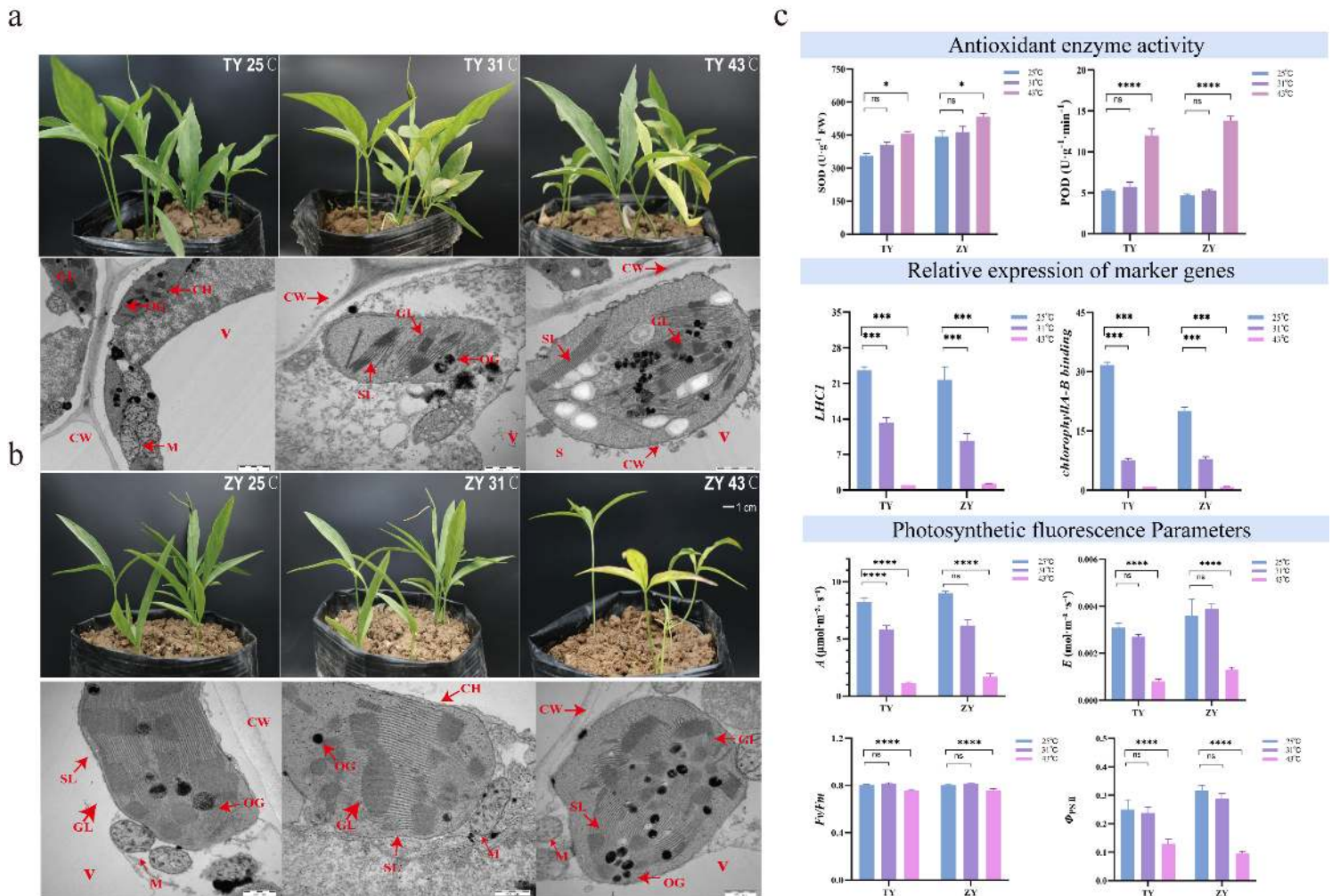


Figure 1

Effects of heat stress on the morphology and physiological parameters of *P. ternata* leaves in response to different heat treatments

(a) Phenotypic changes and leaf ultrastructure of heat stress of TY25, TY31, and TY43; (b) Phenotypic changes and leaf ultrastructure of ZY25, ZY31, and ZY43. Plants were treated with 25°C, 31°C, and 43°C for 12 h. Bar = 1 cm. CH: chloroplasts, CW: cell wall, V: vacuole, M: mitochondrion, OG: osmiophilic particles, GL: granum lamella, SL: stroma lamella, S: starch granule. (c) Physiological changes of *P. ternata* leaves with different heat treatments. Changes in SOD and POD activities in *P. ternata*; Relative expression levels of two marker genes *LHC1* and *chlorophyll A-B binding* in *P. ternata*, determined by qRT-PCR; Changes of photosynthetic gas exchange and chlorophyll fluorescence parameters including *A*, *E*, *Fv/Fm* and Φ_{PSII} after heat treatments in *P. ternata*; Error bars and letters above the columns respectively indicate SE and significant differences at the 0.05 significance level determined by one-way ANOVA.

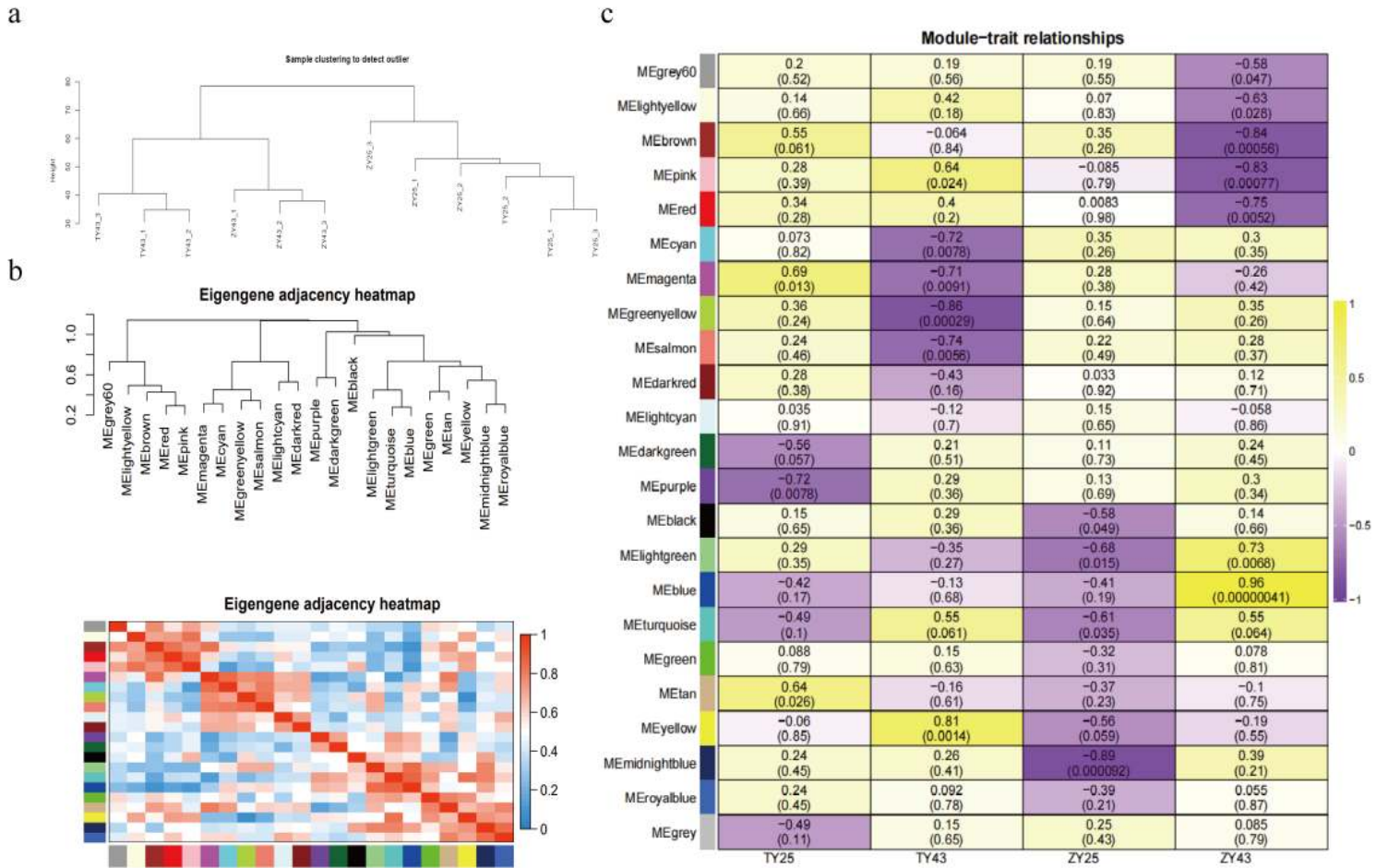


Figure 2

WGCNA analysis of DEGs in heat stress responses of the two leaf types of *P. ternata*

(a) A clustering dendrogram of DEGs. (b) A heatmap illustrates eigengenes' connectivity, providing dissimilarity based on the topological overlap alongside assigned module colors. (c) The module-trait relationships, where each row corresponds to a module and each column corresponds to a specific sample. The Pearson coefficient and its corresponding p -value are reported, with highly positive correlations displayed in dark yellow and highly negative correlations in dark purple. The combination of module-trait marked in a black frame will be chosen for annotation analysis.

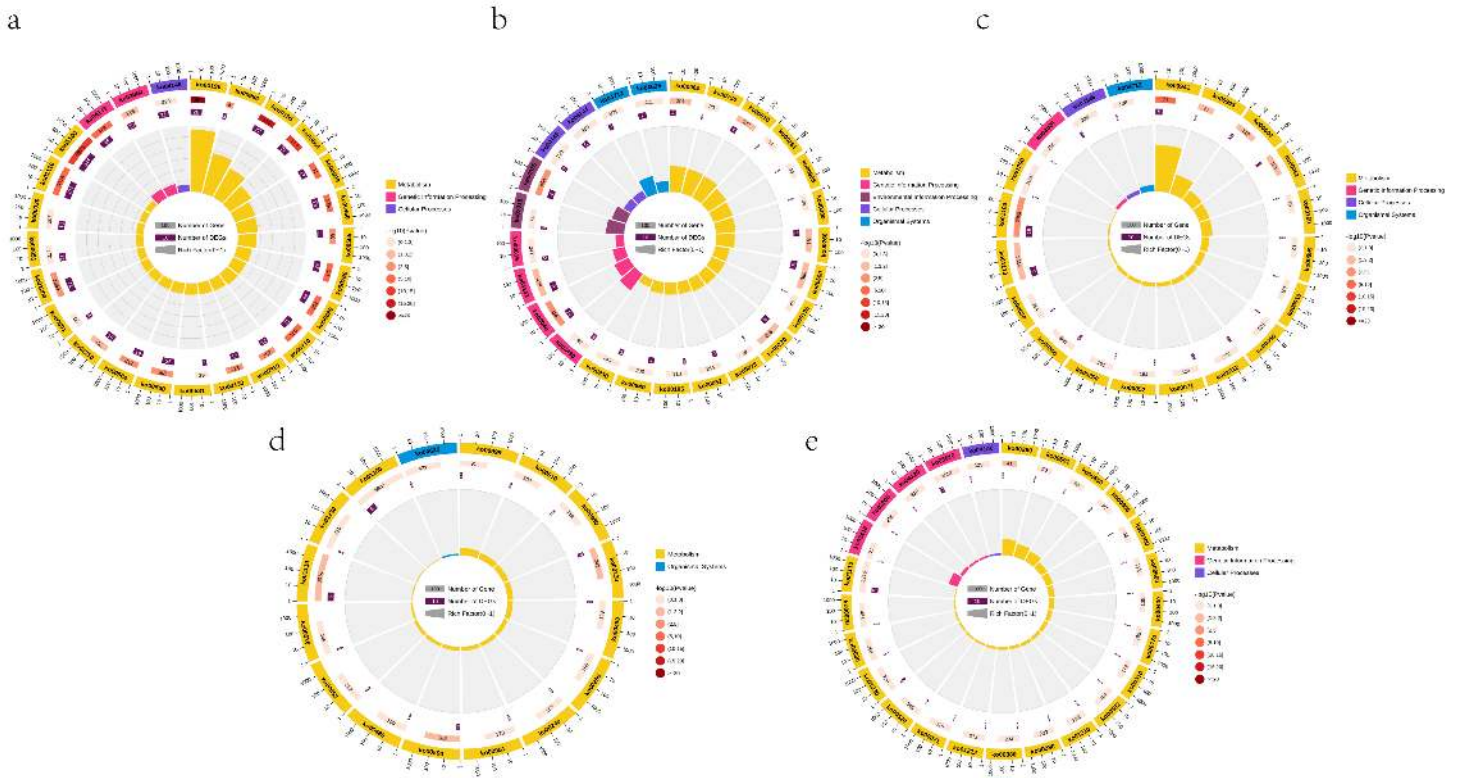
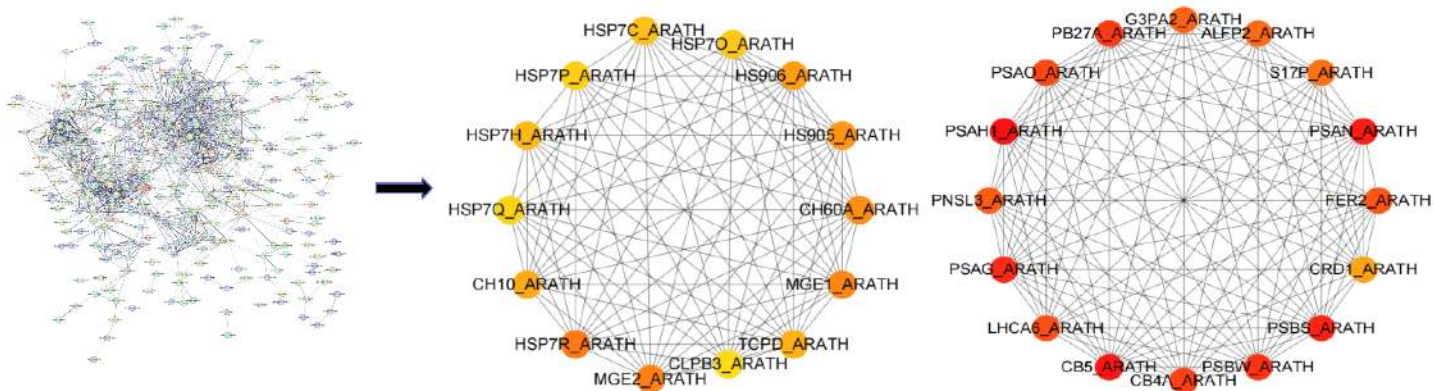


Figure 3

KEGG analysis of DEGs in the obtained five modules

(a) turquoise module; (b) blue module; (c) magenta module; (d) midnight module; (e) salmon module. Circles are viewed from outside to inside. Circle 1 is the pathway enrichment classification, with the number of genes on the outside of the circle on the scale. Circle 2 indicates that the more genes in this category in the background, the longer the bar graph. Circle 3 shows the total number of genes in the foreground. The higher the significance, the darker the red colour. Circle 4 shows the RichFactor value for each pathway-enriched category.

a



b

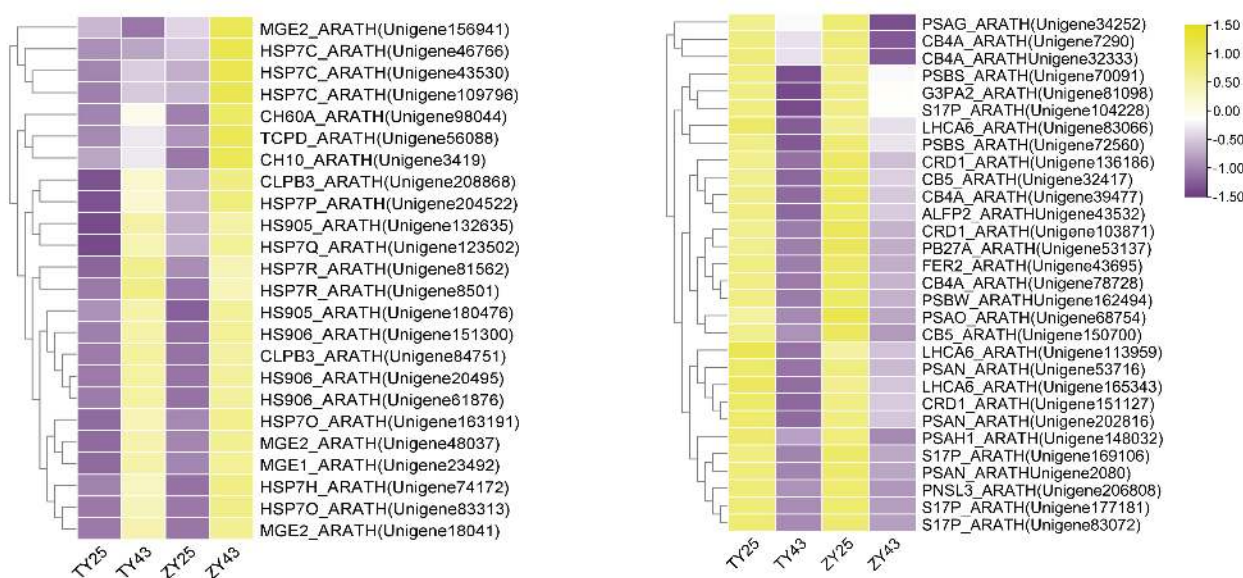


Figure 4

Visualization of the protein–protein interaction (PPI) network and expression patterns of hub genes
 (a) PPI network of 5 interested modules. (b) Heatmap showing the expression profile of 24 hub genes of 5 modules involved in heat stress response and 30 hub genes in photosynthesis pathways. The red nodes present intersection genes.

Supplementary Files

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